Ph.D. Thesis Synopsis

Computational drug discovery for designing novel hTLR4 antagonist and their biological evaluation as anti-inflammatory/anti-proliferative agent

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A Synopsis of Ph.D. Research Work

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Abstract

Toll-Like Receptors (TLRs) are Pattern Recognition Receptor (PRR) and centrally involved in the initiation of the innate and adaptive immune responses. TLRs have shown cellular specificity in terms of ligand recognition, expression and cellular localization in different cell types and tissues, and importantly involved in the pathogenesis of multiple chronic inflammatory diseases. At present, 13 different mammalian TLRs including 11 in human (TLR1-TLR11) and 13 in mice (TLR1-TLR13), with some differences at functional level have been identified. Particularly, TLRs 1 to 11 are conserved in both human and mice, but TLR10 is non-functional in mice, while TLR11 is non-functional in human. In humans, TLRs are expressed on a variety of immune and sentinel cells including myeloid dendritic cells (DCs), monocytes, mast cells, and T and B lymphocytes, synovial fibroblast-like cells and epithelial cells.TLRs are known to have their expression over cell surface, exhibits their response towards bacterial, fungal and protozoan antigens, whereas those TLRs have expression in cytoplasm display response towards the viral, microbial, and host nucleic acids. Indeed, TLRs are also profoundly expressed on various types of cancers and associated with cellular response for cell survival. Toll-like receptor 4 (TLR4) is a member of Toll-Like Receptors (TLRs) family that serves as a receptor for bacterial lipopolysaccharide (LPS). TLR4 alone cannot recognize LPS without aid of co-receptor myeloid differentiation factor-2 (MD-2). Binding of LPS with TLR4 forms a LPS–TLR4–MD-2 complex and directs downstream signaling for activation of immune response, inflammation and NF-κB activation. Activation of TLR4 signaling is associated with various inflammatory pathophysiological proveniences. Therefore, targeting protein–protein interaction (PPIs) in TLR4–MD-2 complex formation could be an attractive approach for identifying TLR4 inhibitor.The aim of the present study was to explore the publically available chemical compound library in search of novel lead molecules as potential TLR4 antagonists. To achieve aim of the study all the three methods of computational drug discovery namely ligand-based, structure-based and pharmacophore based approach were applied. In

ligand-based approach, caffeic acid based analogs have been designed that were subjected to molecular modeling, docking and dynamics study followed by their synthesis. In structure-based approach, we screened world subset of ZINC15 database (3447 compounds) to reposition FDA approved drugs with some other indication against our target protein by implementing the methods of molecular docking and dynamics followed by their *in silico* investigation. After applying structure-based approach, we identified approximately 50 compounds from structure guided *in silico* drug repurposing predicted to be potential TLR4 signaling inhibitors via targeting MD-2 protein. In pharmacophore-based approach we have shown the potential of pharmacophore mapping based approach of computational drug discovery in retrieving the information about the hot spot residues and their pharmacophoric features at both primary (TLR4–MD-2) and dimerization (MD-2–TLR4*) protein-protein interfaces in TLR4–MD-2–LPS homodimer complex.Pharmacophore query was used to screen ZINC[12] database for creation of focused library containing 5519 unique compounds which were subjected to molecular docking and dynamics study. After applying molecular docking in combination with ADMET filtration the study has resulted in selection of hit list of 25 molecules at the end that are predicted to have all the properties to become better lead molecules as well as good PPI inhibitors. Selective virtually screened compounds from structure-based and pharmacophore based approach were obtained for their *in vitro* investigation. All the synthesized and purchased compounds from three approaches were subjected to *in vitro* investigation to confirm their inhibitory potential as anti-proliferative and anti-inflammatory agents against the LPS induced inflammatory responses.

1. Introduction

Toll-like receptors (TLRs) are evolutionary conserved Pattern Recognition Receptor (PRR) and widely expressed on the surface of the immune cells [1]. Toll-like receptors have an imperative role in immunity, neurogenesis and developmental process across the eukaryotic species [2]. All the species have these conserved biological functions. TLRs are a most conserved family of receptors and were first identified in 1985 by Nüsslein-Volhard *et al.* in *Drosophila*. This discovery was based on the roleof *Drosophila* Toll (dToll) in development of embryonic dorsoventral polarity [3-5]. Later, the role of dToll was identified in inducing innate immune responses in adult *Drosophila* against fungal infection [6-8]. Indeed, it was identified that TLRs are also involved in the activation of immune responses and transcription factor NF-κB signaling pathways in many species [9, 10].TLRs are highly susceptible towards infection [11]. At present, 13 different mammalian TLRs (11 in human and 13 in mice) have been identified, but they have some differences at the functional level[12, 13].

In humans, TLRs are classified into five subfamilies (TLR3, TLR4, TLR5, TLR2 and TLR9) on the basis of comparative study of amino acid sequences [14]. The TLR2 subfamily comprises of TLR1, TLR2, TLR6, and TLR10; the TLR9 subfamily is comprises of TLR7, TLR8, and TLR9. TLRs 1, 2, 4, 5, 6 and 10 are known to be expressed on the cell surface, whereas TLRs 3, 7, 8, and 9 are known to have their expression intracellularly [15]. TLRs, that are known to be present on cell surface, typically show their response towards bacterial or fungal antigens, whereas TLRs present in cytoplasm exhibits response towards the viral or microbial nucleic acids [16-19]. TLRs show their response against the varied forms of microbial products, cellular mediators derived from damaged tissue or endogenous molecules, those are also known as danger-associated molecular patterns (DAMPs), and triggers multiple signal transduction pathways in the similar fashion as they are activated by the pro-inflammatory cytokine IL-1, as the cytosolic portions of IL-1R and TLRs are known to be homologous and possess a common intracellular TIR domain which is known to be involved in the initiation of signaling [20].

Structurally, TLRs are transmembrane domain receptors comprises three domains including a leucine-rich region (LRR), transmembrane and intracellular

Toll/interleukin-1 receptor (TIR) domain. The leucine-rich hydrophobic domain consists of 19-25 leucine-rich motifs responsible for binding of microbe-associated molecular patterns (MAMPs) or pathogen-associated molecular patterns (PAMPs) or endogenous ligands such as nucleic acids, lipids, proteins, and lipoproteins etc. Transmembrane domain interplays between leucine-rich and intracellular Toll/interleukin-1 receptor (TIR) domain[21].The TIR domain interacts with adaptor proteins such as MyD88, Mal, TRIF, TIRAP, and TRAM etc. to activate downstream NF-κB signaling pathways [22-25]. Although, different TLRs can activate distinct cellular responses by activation of NF-κB, AP-1, and MAPK signal transduction pathway through the association of different adaptor molecules[26, 27].

TLR4 is the key pattern recognition receptors that are associated with chronic inflammatory disorders and setting tumor microenvironment. TLR4 can recognize both PAMPs and DAMPs. Activation of TLR4 signaling is initiated by ligand recognition, dimerization, and recruitment of adaptor proteins.TLR4 is known to be associated with the inflammatory responses via triggering several signaling pathways that may lead to chronic inflammatory disorders such as sepsis [28], nephrotoxicity [29], cancer [15, 30-34], rheumatoid arthritis [35], etc. The inflammatory environment can fosters tumor growth, angiogenesis and metastasis. In recent part many reports reveal that expression of TLR4 and activated signaling is associated with inflammation and progression of tumorigenesis [36].

Cancer cells are aligned in dynamic interaction with the host's immune cells (immune infiltrates such as DCs, Macrophages, Bcells) for immune evasion, tumor cell survival, growth, and metastasis. Recent reports suggest that hTLR4 is not only expressing in immune cells, but also expressed in variety of cancer cells.TLR4 play crucial role in facilitating immune escape mechanism of tumorigenesis leading to various types of cancer such as breast cancer, cervical cancer, urothelial bladder cancer (UBC), pancreatic cancer, digestive cancers (gastric, colorectal, hepato-cellular carcinoma, gallbladder cancer and gastric mucosa associated lymphoid tissue (MALT) lymphoma) etc.[37-41]. The activation of TLR4 signaling promotes tumor microenvironment with the aid of NF-kB activation, pro-inflammatory cytokines production to modulate the

immune response and develop resistance against programmed cell death by apoptosis via increasing the expression of anti-apoptotic proteins that allow for cell survival mechanism[42, 43].

Several studies have been performed to date for the evaluation of clinical significance of TLR4 agonist in cancer therapeutics and suggest that TLR4 activation via agonism could have potential as cancer vaccination, conventional chemotherapeutics and other therapeutic approaches. Several agonists for TLR4 have been reported so far along with their clinical significance in cancer therapy[37, 44]. However, a growing body of evidences are also there for the suppression and evasion of vaccine induced antitumor immunity by tumor cells. It is remaining to understand and resolve that what level of TLR4 activation is needed to overcome immune escape mechanism of tumorigenesis as there are several studies in support of facilitation of immune escape mechanism by TLR4 stimulation resulting in chronic inflammation and cancer cell proliferation. In recent past it has been suggested that combinations of synergistic therapies could be helpful in achieving the full cancer therapeutic potential of TLR4 activation through agonist[14]. Different studies have demonstrated that therapeutic interventions for TLR4 stimulation via agonism may behave as a double-edged sword as both the positive as well as negative effects of TLR4 stimulation have been seen on cancer development or treatment. Although, controlling the negative effect of TLR4 stimulation while harnessing the beneficial effects during the cancer regimen is still a challenge for researchers working in the field of cancer immunotherapy via targeting TLR4 through agonistic approach[45].To overcome the side-effects and after-effect of agonist, various combination therapies are also in clinical application[46].Agonistic therapeutics towards hTLR4 stimulation may be more successful in vaccination against tumor/cancer, but is not of very use in late stages, whereas antagonist could be better therapeutics at the advanced stages of cancer[47].

Integration of computational approaches for drug designing is one of the promising tool to find lead molecules. In continuation of recent development of drug discovery approaches Murgueitio et al. have discussed the three major applicable areas of computational approaches, which can help improve the speed of discovery process in

the context of TLR modulators[48]. These include (i) exploration of TLR structure and activation mechanism; (ii) finding the possibilities of TLR modulation mechanism; (iii) discovery of new lead molecules, which may be active as agonist or antagonist of TLRs[47].The same application areas are also applicable to the discovery of hTLR4 modulators. In the recent past, computational methods and tools facilitated the deep investigation of structural and functional dynamics of TLR4 in humans, especially when it comes to the protein-protein interaction betweenhTLR4 and MD-2, which provides a rational approach to design novel PPI modulators. Mechanistic insights into binding and dimerization mechanisms by applying computational methods provide a systemic approach to discover more specific and selective small molecules as novel hTLR4 signaling modulators[48].

Most of the small molecule modulators of TLR4 are either designed by mimicking the natural ligands or by high throughput screening (HTS) and were not co-crystallized with the receptor. Although several crystallographic structures of the extracellular domain of hTLR4, hTLR4–MD-2 complex, and MD-2 have been solved in last one decade, but there is still a limitation of co-crystallized receptor-synthetic ligand conformations. Computational methods such as molecular modeling, molecular docking/dynamics and QSAR provide the better resolution to understand the underlying mechanism of molecular action at the atomic level and in-depth understanding to rationalize the structure-activity relationship (SAR) of molecules for further lead optimization[47].In the end of the lead optimization step, only those derivatives with the very best, experimentally confirmed, binding affinity will be retained and find their way into the next step. Recently, Regidor et al. have compiled all the virtual screening campaigns that were successfully applied in the discovery of TLR modulators with agonistic and antagonistic activity[49].Computer-aided dug design may also establish molecules as promising drug candidates that would never have been tested without computer-based methods or their initial unavailability in synthesized form. It is an important to address that conventional drug development necessitates enormous cost, efforts, time and limited chance of successful leads of new drug candidate. In essence, computer-aided drug design approaches helps to predict properties and actions of

chemical compounds by a variety of techniques, so that molecules that are unlikely to experience the desired effect on the chosen molecular target can be cast aside. Beyond the above drug repositioning or repurposing is an emerging process of finding new uses of an old and existing drugs that could be either disease or target specific[50]. Drug repurposing help to minimize the risk of failure during preclinical development and optimization process hence it reduces the overall cost of drug development[51].

Although, advancement of science has an era of "big data" in the field of biological and chemical information [52]. Still we are very far from the linking of all the chemistries of compounds with the biologies of macro-molecules due to the limited availability of suitable information and molecular mechanism of action. Drug can be repurposed in two direction, either by finding association of drug with the target and target with disease or to identify a disease associated target followed by identification of the drugs that can be repositioned for the desired target. Computational methods of drug repurposing can be applied in both the directions to meet the goal as well as can be helpful in filling the gap due to lack of information. Several successful applications have been made in the repositioning of drugs with the help of computational methods.

2. Hypothesis

hTLR4 is one of the most potent therapeutic targets among the other TLRs. TLR4 is a member of Toll-Like Receptors (TLRs) family that serves as a receptor for bacterial lipopolysaccharide (LPS). But, importantly TLR4 alone cannot recognize LPS without aid of co-receptor myeloid differentiation factor-2 (MD-2). Binding of LPS with TLR4 forms a LPS–TLR4–MD-2 complex and directs downstream signaling for activation of immune response, inflammation and NF-κB activation. Activation of TLR4 signaling leads to setup of inflammation and tumor micro-environment resulted provenience of chronic pathophysiology and cancer. Therefore, targeting to design TLR4 antagonist or protein–protein interaction inhibitors (PPIs) for TLR4–MD-2 complex could be an attractive therapeutic approach and lead of drug discovery. The aim of present study was directed to identify small molecule as TLR4 antagonist or PPI inhibitors (SMPPIIs) using different approaches of computational drug discovery to identify lead moleculesfor

therapeutic value.There was following objectives were proposed to achieve aim of the study.

3. Objectives

The major objectives of the proposed study are as follows:

1. To perform ligand, structure and pharmacophore based virtual screening approaches for screening large chemical compound libraries for the identification of small lead molecules having antagonistic/ inhibitory activity against hTLR4.
2. Molecular docking/dynamic simulation studies of virtually screened compounds.
3. *In silico* evaluation of virtually screened compounds on the basis of their molecular properties/ descriptors.
4. Synthesis, characterization and in-vitro evaluation of screened compounds in inflammatory/cancer cells by measuring inhibition of cell proliferation.
5. To determine effects of hTLR4 antagonist on inhibition of inflammatory cytokines (IL1b, IL6 and TNF-a) and NF-kB activation.

4. The following salient findings were achieved from the present study:

Objective 1: To perform ligand, structure and pharmacophore based virtual screening approaches for screening large chemical compound libraries for the identification of small lead molecules having antagonistic/ inhibitory activity against hTLR4.

The promising hits were achieved by applying the methods of ligand, structure and pharmacophore-based virtual screening approaches:

a) Ligand-Based Approach

In ligand-based approach, molecular modelling and docking were used to design five analogs (N1-N5) of caffeic acid (CA). These analogswere predicted as potential hTLR4 binders after *in silico* investigation. Caffeic acid and their designed analogs were modelled with the help of Chemaxon toolkit. During molecular docking study it was observed that all these analogs bind within the pocket of MD-2 protein, which reflects the idea that these compounds may inhibit the TLR4 signaling via occupying the ligand binding space at MD-2 pocket. The study also reveal that some of these analogs

maypossibly have higher binding affinity towards apo MD-2 (PDB id: 2E59) as compared to TLR4-MD-2 complex (PDB id: 3FXI). Binding affinities were estimated for caffeic acid analogs with respect to co-crystallized antagonist (eritoran) in complex with hTLR4-MD-2 (PDB id: 2Z65). ADME modelling was also performed for designed analogs to compare it with reference ligand as well as other known inhibitors of TLR4. The results highlight that resulted compound from ligand based approach may be promising candidate to disrupt TLR4 mediated inflammatory signaling response.

b) Structure-Based Approach

The aim of the present study was to reposition the previously approved drug with other indication for our target protein i.e. TLR4–MD-2 complex. To achieve the goal, we selected world subset of ZINC[15] database as a compound library. The subset contains 3447 compounds at the time of study that were approved by major jurisdictions including FDA. Molecular docking was used to reposition the drug within the binding pocket of the target protein. More than 50 compounds were identified as potential binders of MD-2 protein. These compounds seem to bind with MD-2 through hydrophobic interactions. Interestingly, most of these compounds are previously indicated as anti-inflammatory, anti-histamine, anti-allergic, and anti-cancer agents. The study proposes that these compounds may be novel candidate for targeting TLR4 signaling

c) Pharmacophore-Based Approach

The aim of present study was directed to identify small molecule PPI inhibitors (SMPPIIs) using pharmacophore mapping-based approach of computational drug discovery. To achieve the aim we retrieved the information about the hot spot residues and their pharmacophoric features at both primary (TLR4–MD-2) and dimerization (MD-2–TLR4*) protein–protein interaction interfaces in TLR4–MD-2 homo-dimer complex using *in silico* methods. Pharmacophoric features based queries were constructed for the target complex. The queries were used to search ZINC[12] database for creation of focused library (5519 compounds). The library was subjected to virtual screening by applying the methods of molecular docking. The study has resulted in 25 promising lead candidates after pharmacokinetics/ pharmacodynamics predictions. These compounds were seems promising to restrict TLR4–MD-2 protein–protein

interaction selectively. *In silico* off-target profiling over the virtually screened compounds was also performed with an intension to explore other possible molecular targets that may interfere with the overall efficacy either by promoting or inhibiting the candidate molecules' desired response. Two of the virtually screened compounds (C11 and C15) were predicted to have an inhibitory concentration in μM range after HYDE assessment. Molecular dynamics simulation study performed for these two compounds in complex with target protein confirms the stability of the complex.

Objective 2: Molecular docking/ dynamic simulation studies of virtually screened compounds.

Molecular docking study of some of the virtually screened compounds (obtained in objective 1) aided to explore the binding potential, ligand efficacy, residues specific binding potential (selectivity), etc. of newly identified small leads towards hTLR4 and MD2. Molecular dynamics simulation(MDS) was performed to confirm the stability of the protein–ligand-docked conformation in a dynamic system. For the same purpose best docked poses of some highest scoring ligands in complex with the target protein were used for building a system for MDS. After MDS, the RMSD values were used to confirm the stability of the complex in a dynamic environment. Interestingly, several compounds that were screened as actives via LBDD, SBDD, and PBDD, seem to have ability to form stable complex with target protein. Molecular dynamics simulation study supports the molecular docking results in terms of binding conformation and stability of the receptor–ligand complex. Most of the amino acid residues that are found to be involved in hydrogen bond interaction during MDS were predicted to be crucial residues for both protein-protein interaction as well as ligand recognition. Thus, the study suggests that these compounds have the potential to target important residues even during dynamic condition before getting stable with the target protein.

Objective 3:*In silico* evaluation of virtually screened compounds on the basis of their molecular properties/ descriptors.

In silico evaluation of virtually screened compounds were performed in order to explore their drug likeness by performing *in silico* ADME/T (Absorption, Distribution,

Metabolism, Excretion and Toxicity) analysis. ADMET analysis and Physico-Chemical profiling were performed on the basis of their molecular properties/descriptors such as lipophilicity, polar solvent accessibility, molecular weight, no. of hydrogen bond donor/acceptor etc. Prediction of ADMET properties of newly discovered lead molecules may help in reducing the risk of clinical failure and drug safety issues related to human health and environmental hazards in later stages of drug development. Drug-likeness and possible side effects such as mutagenic, tumorigenic, reproductive and irritant effects were calculated. These efforts may be helpful in minimizing the cost of experimentation over the lead molecules that have higher chances of failure at later stages due to poor pharmacokinetics/pharmacodynamics and are associated with higher risk. The study also helps to understand the weaknesses of lead molecules that may guide through finding the ways to improvise these leads in terms of their potency. *In silico* study reveal that all the designed caffeic acid analogs are suitable for human intestinal absorption, whereas some of the known inhibitors of hTLR4 such as Eritoran, IAXO-101, IAXO-103, Polymyxin B sulphate are predicted negative for human intestinal absorption, which may be the one of the possible causes of poor efficacy of these inhibitors. Caffeic acid and its analogs are also predicted not to be substrate for p-gp which will help to reduce the problem of multi drug resistance (MDR) associated with some of the present inhibitors of hTLR4 such as Eritoran, IAXO-101, IAXO-102, IAXO-103, CRX-526, Polymyxin B sulphate as these inhibitors of TLR4 were predicted to be substrate of p-gp. Most of the compounds obtained after structure guided *in silico*drug repurposing were found to be suitable for human intestinal absorption and crossing blood brain barrier. Most of these compounds are also not the substrate for p-gp and thus avoid the problem of development of drug resistance. The compounds selected as virtually active PPI inhibitors after pharmacophore based virtual screening also have both positive and negative values for human intestinal absorption and for crossing blood brain barrier. Most of the compounds obtained from all the three approaches mentioned above are also have predicted IC50 (pIC50) value for hERG protein within the tolerability range i.e. below 5. Most of these compounds also have inhibitory activity for 2C9 within the range of tolerance i.e. below 6. Most of these compounds also have 2D6

binding affinity from low to medium which is also under the category of acceptable lead molecules.

Objective 4: Synthesis, characterization and in-vitro evaluation of screened compounds in inflammatory/cancer cells.

The caffeic acid derivatives (N1-N5) obtained from ligand based approach were synthesized and confirmed by ^{1}H NMR and ^{13}C NMR. The compounds obtained from virtual screening from pharmacophore (C1) and structure-based (C2-C6) approaches were obtained commercially. The effect of these compounds were tested for NO and ROS production in Raw 264.7 cells. The results reveal that all the identified lead molecules showed significant inhibition in NO production in Raw 264.7 cells. Further, cell viability was examined by MTT assay, which show that these compounds are restricting cell proliferation.

Objective 5: To determine effects of hTLR4 antagonist on inhibition of inflammatory cytokines (IL1b, IL6 and TNF-a) and NF-kB signaling response.

LPS induces pro-inflammatory cytokines synthesis as well as their release. Here, the effect of identified hTLR4 antagonist (C1-C6) and (N1-N5) were examined on release of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) upon pre-treatment of these compounds with subsequent exposure of LPS for 24 hrs. in THP-1 cells (acute monocytic leukemia cells). Our results showed that the THP-1 cells exposed with LPS (100 ng/ml) significantly increases IL-1β, IL-6 and TNF-α secretion. However, pre-treatment with these compounds showed significant reduction in IL-1β, IL-6 and TNF-α production. The effect of these compounds was noticed similar and more promising as compared to known inhibitor of TLR4 (TLR4-IN-C34). Next, effects of these compounds were monitored on regulation of cytosolic to nuclear translocation of NF-κB, which is a transcription factor and plays a major role in the regulation of inflammatory condition. Under normal condition, NF-κB retains in the cytoplasm, but upon activation it translocate to the nucleus and activates various sets of genes. Here, NF-κB activation was monitored under microscopic examination using GFP-tagged p65 in MCF-7 cells. The results show that the cells that were treated with LPS have prominent expression of p65

in the nucleus. However, cells were treated with these selected compounds prominently restrict cytosolic to nuclear translocation of p65 even in the presence of LPS. Thus, these results suggest that these compounds efficiently inhibit pro-inflammatory cytokines and NF-κB activation in response to LPS (TLR4 agonist).

5. Conclusion

This study explore a small molecule as TLR4 antagonist or PPI inhibitors (SMPPIIs) using different approaches of computational drug discovery to identify lead molecules for therapeutic value. The present study has considered all the parameters including ADME/T filtration, oral bioavailability, crossing the blood brain barrier (BBB), reduced possible side effects and environmental hazards that may limit failure during hit to lead identification and lead to drug conversion during experimental and pre-clinical/clinical trials.

- Interestingly, some compounds were found to have novel molecular scaffolds that were not reported in previously known TLR4 inhibitors.
- The compounds obtained as a result of *in silico* drug repurposing were previously reported to have anti-inflammatory, anti-cancer, anti-histamine and anti-allergic activity. The study suggests that these compounds may have these activity via targeting TLR4 signaling.
- It may be concluded from this analysis that cyclic and aromatic ring attached directly to α, β- unsaturated carbonyl group of caffeic acid through amide linkage play an important role in increasing its ability to cross the blood brain barrier.
- After *in vitro* investigation, all the compounds (C1-C6 and N1-N5) seem promising in inhibiting LPS induced NO production. These compounds also inhibited secretion of pro-inflammatory mediators (TNF-α, IL-6, and IL-1β) and NF-κB activation and cell proliferation.

Thus, taken together our study revealed novel TLR4 antagonists that may inhibit TLR4 signaling by binding as ligand or PPI inhibitors. The candidates may me lead molecules for developing new drug candidate.

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List of Publications

1. **Vinita Mishra**& Chandramani Pathak, "Human Toll-Like Receptor 4 (hTLR4): Structural and functional dynamics in cancer", *International Journal of Biological Macromolecules* 122:425-451 (2019)(IF: 3.9)**(Review Article).**
2. **Vinita Mishra**& Chandramani Pathak, "Structural insights into pharmacophore-assisted *in silico* identification of protein–protein interaction inhibitors for inhibition of human toll-like receptor 4–myeloid differentiation factor-2 (hTLR4–MD-2) complex", *Journal of Biomolecular Structure and Dynamics,* 1-24 (2018) (doi: 10.1080/07391102.2018.1474804.) (IF: 3.1)**(Research Article).**

List of Presentations

1. **VinitaMishra,**Khushbu Priyadarshi and Chandramani Pathak, "Polypharmacological Study of Gallic Acid Through Identifying Molecular Targets: An *In silico* Analysis", 4thNIRMA Institute of Pharmacy International Conference on "Innovation in Pharmaceutical Research by Interdisciplinary Approach (NIPiCON-2018)", organized by Institute of Pharmacy, NIRMA University, Ahmedabad, 23rd – 25th January, 2018 **(Poster Presentation).**
2. **Vinita Mishra**, Neha Kapoor and Chandramani Pathak, "Molecular modelling and docking of caffeic acid and its amide derivatives to explore as anti-inflammatory agents via targeting human TLR4 (hTLR4)" **(Abstract)**, International Conference on Advancing Green Chemistry: Building a Sustainable Tomorrow, organized by Green Chemistry Network Centre, Department of Chemistry, University of Delhi & Hindu College, University of Delhi, 3rd–4th October, 2017 **(Oral).**
3. **Vinita Mishra** and Chandramani Pathak*, "*In silico* analysis of novel chemical modulators for human Toll-Like Receptor 4 (hTLR4) as an antagonist" **(Abstract)**, International conference on Drug Design (ICDD 2017), organized by Schrodinger at JNU, 7th–9th April, 2017 **(Poster).**
4. **Vinita Mishra** and Chandramani Pathak*, "Pharmacophore assisted *in silico* discovery of small molecules PPI inhibitors (SMPPIIs) for Targeting TLR4–MD-2

Interaction", 2nd National Conference "Emerging Trends in Drug Discovery, Development and Molecular Targets for Cancer Research" organized by Institute of Pharmacy, NIRMA University, Ahmedabad, 24th–25th January, 2017 **(Poster).**

5. **Vinita Mishra** and Chandramani Pathak*, "Pharmacophore based *in silico* approach for inhibition of protein -protein interaction specially focused to TLR 4–MD-2 complex" **(Abstract)**, 6th International Translational Cancer Research Conference "Prevention and Treatment of Cancer: Hypes & Hopes" organized by the Gujarat Cancer & Research Institute (GCRI), International Society for Translational Cancer Research, the Gujarat Cancer Society, 4th–7th February, 2016 **(Poster).**
6. **Vinita Mishra** and Chandramani Pathak*, "Structural analysis of Toll-Like Receptor 4 (TLR4) as anti-inflammatory target: An *in silico* study" **(Abstract)**,Ramanbhai Foundation 7th International Symposium on Current Trends in Pharmaceutical Sciences "Advances in New Drug Discovery And Development" organized by Zydus Research Centre, Ahmedabad, India, 2nd–4th February, 2015 **(Poster).**

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